10/089214 JC05 Rec'd PCT/PTO 25 MAR 2002

*FORM PTO-1390 (REV 11-2000) U S DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. § 371

ATTORNEY'S DOCKET NUMBER: 246152016400

U S APPLICATION NO. (If known, see 37 CFR 1.5): 10/089214

INTERNATIONAL APPLICATION NO	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/NL00/00635	September 8, 2000	October 27, 1999

TITLE OF INVENTION: **PROCESS FOR THE PREPARATION OF A DIPEPTIDE AND INTERMEDIATE PRODUCT IN SUCH A PROCESS**

APPLICANT(S) FOR DO/EO/US: **Wilhelmus Hubertus Joseph BOESTEN, , Quirinus Bernardus BROXTERMAN, Marcus Joseph Maria PLAUM**

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. ☒ This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. ☐ This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U S C. 371.
3. ☒ This is an express request to begin national examination procedures (35 U.S.C. 371(f)). The submission must include items (5), (6), (9) and (21) indicated below.
4. ☐ The US has been elected by the expiration of 19 months from the priority date (PCT Article 31).
5. ☒ A copy of the International Application as filed (35 U.S C. 371(c)(2))
   a. ☐ is attached hereto (required only if not communicated by the International Bureau).
   b. ☒ has been communicated by the International Bureau.
   c. ☐ is not required, as the application was filed in the United States Receiving Office (RO/US).
6. ☐ An English language translation of the International Application as filed (35 U S C. 371(c)(2)).
   a. ☐ is attached hereto.
   b. ☐ has been previously submitted under 35 U S C. 154(d)(4)
7. ☒ Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3)).
   a. ☐ are attached hereto (required only if not communicated by the International Bureau)
   b. ☐ have been communicated by the International Bureau.
   c. ☐ have not been made; however, the time limit for making such amendments has NOT expired
   d. ☒ have not been made and will not be made.
8. ☐ An English language translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3))
9. ☒ An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4))
10. ☐ An English language translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U S C 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

11. ☐ An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. ☒ An assignment document for recording A separate cover sheet in compliance with 37 CFR 3 28 and 3.31 is included.
13. ☒ A FIRST preliminary amendment
14. ☐ A SECOND or SUBSEQUENT preliminary amendment
15. ☒ A substitute specification. (copy)
16. ☐ A change of power of attorney and/or address letter.
17. ☐ A computer-readable form of the sequence listing in accordance with PCT Rule 13ter.2 and 35 U.S.C 1.821 - 1.825
18. ☐ A second copy of the published international application under 35 U S C. 154(d)(4).
19. ☐ A second copy of the English language translation of the international application under 35 U.S.C. 154(d)(4)
20. ☒ Other items or information (1) Certified copy of priority document No 1013404 filed in The Netherlands and (2) return receipt postcard

CERTIFICATE OF MAILING BY "EXPRESS MAIL"

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Marian Christopher

sd-83429

10/089214 JC13 Rec'd PCT/PTO 25 MAR 2002

U.S. APPLICATION NO (if known, see 37 CFR 1.5)	INTERNATIONAL APPLICATION NO	ATTORNEY'S DOCKET NUMBER
10/089214 To be Assigned	PCT/NL00/00701	246152016500

21. ☒ The following fees are submitted:

BASIC NATIONAL FEE (37 CFR 1.492(a)(1)-(5)):

Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO and International Search Report not prepared by the EPO or JPO.....$1,040.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but International Search Report prepared by the EPO or JPO.....$890.00

International preliminary examination fee (37 CFR 1.482) not paid to USPTO but international search fee (37 CFR 1.445(a)(2)) paid to USPTO.....$740.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO but all claims did not satisfy provision of PCT Article 33(1)-(4).....$710.00

International preliminary examination fee (37 CFR 1.482) paid to USPTO and all claims satisfied provisions of PCT Article 33(1)-(4).....$100.00

	CALCULATIONS PTO USE ONLY	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$890	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).	$	

CLAIMS	NUMBER FILED	NUMBER EXTRA	RATE		
Total claims	13 - 20 =	0	x $18.00	$	
Independent claims	4 - 3 =	1	x $84.00	$84	
MULTIPLE DEPENDENT CLAIM(S) (if applicable)			+ $280.00	$	

TOTAL OF ABOVE CALCULATIONS =	$974	
☐ Applicant claims small entity status. See 37 CFR 1.27. The fees indicated above are reduced by ½.	$	
SUBTOTAL =	$	
Processing fee of **$130.00** for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +	$	
TOTAL NATIONAL FEE =	$974	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). **$40.00 per property** +	$40	
TOTAL FEES ENCLOSED =	$1,014	
	Amount to be refunded:	**$***
	charged:	**$1,014**

a. ☐ A check in the amount of $* to cover the above fees is enclosed.

b. ☒ Please charge my **Deposit Account No. 03-1952** in the amount of $1,014 to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees that may be required, or credit any overpayment to **Deposit Account No. 03-1952**. A duplicate copy of this sheet is enclosed.

d. ☐ Fees are to be charged to a credit card. **WARNING:** Information on this form may become public. Credit card information should not be included on this form. Provide credit card information and authorization on PTO-2038.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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SIGNATURE

Carolyn A. Favorito
Registration No. 39,183

sd-83429

10/089214

PATENT

Docket No. 246152016400

Client Reference 3956US/CNT/1/

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CERTIFICATE OF MAILING BY "EXPRESS MAIL"

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Marian Christopher

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In the application of:

Wilhelmus BOESTEN et al.

Serial No.: To be Assigned

Filing Date: Herewith

For: PROCESS FOR THE PREPARATION OF A DIPEPTIDE AND INTERMEDIATE PRODUCT IN SUCH A PROCESS

Examiner: To be Assigned

Group Art Unit: To be assigned

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Dear Sir:

Prior to examination, please amend the application as follows:

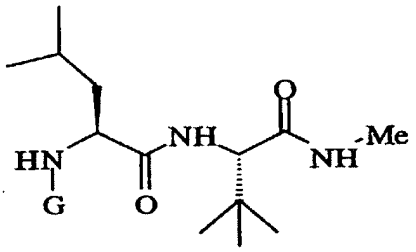
sd-83439

AMENDMENT

In the Claims:

Please replace original claims 1-13 with the following claims:

1. A process for the preparation of a dipeptide of formula 1 comprising



(1)

coupling N-protected L-leucine to L-*tert.*-leucine-N-methylamide in the presence of an activating agent, wherein G is a protective group that is a formyl group.

2. The process according to claim 1 in which the L-*tert.*-leucine-N-methylamide has an enantiomeric excess greater than 98%

3. The process according to claim 1 in which the N-formyl-L-leucine has an enantiomeric excess greater than 98%.

4. The process according to claim 1 further comprising subjecting the N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide obtained to one or more crystallizations.

5. The process according to claim 1 further comprising deformylating the dipeptide obtained.

6. The process according to claim 5 further comprising subjecting the L-leucyl-L-*tert.*-leucine-N-methylamide obtained to one or more crystallizations.

7. The process according to claim 5 further comprising coupling the L-leucyl-L-*tert*.-leucine-N-methylamide to a substituted or nonsubstituted α-mercaptocarboxylic acid to form the corresponding N-α-optionally substituted mercaptocarboxyl-L-leucyl-L-*tert*.-leucine-N-methylamide.

8. A compound which is N-formyl-L-leucyl-L-*tert*.-leucine-N-methylamide.

9. A composition comprising the N-formyl-L-leucyl-L-*tert*.-leucine-N-methylamide defined in claim 8 wherein an enantiomeric excess is present of the N-terminal amino acid in the dipeptide of more than 80%.

10. The composition according to claim 9 wherein the enantiomeric excess of the N-terminal amino acid in the dipeptide is more than 98%.

11. The composition according to claim 9 wherein a diastereomeric excess is present of more than 80%.

12. The composition according to claim 11 with a diastereomeric excess of more than 98%.

13. A pharmaceutical composition comprising N-formyl-L-leucyl-L-*tert*.-leucine-N-methylamide according to claim 8 and a pharmaceutically acceptable excipient.

REMARKS

Attached hereto is a marked-up version of the changes made to the claims by the current amendment. The attached page is captioned "**Version with markings to show changes made**".

The above changes were made to conform the claims to U.S. patent practice. No new matter has been added.

In the unlikely event that the transmittal letter is separated from this document and the Patent Office determines that an extension and/or other relief is required, applicant petitions for any required relief including extensions of time and authorizes the Assistant Commissioner to charge the cost of such petitions and/or other fees due in connection with the filing of this document to **Deposit Account No. 03-1952** referencing docket no. 246152016400. However, the Assistant Commissioner is not authorized to charge the cost of the issue fee to the Deposit Account.

Respectfully submitted,

Dated: March 25, 2002

By: _____

Carolyn Favorito
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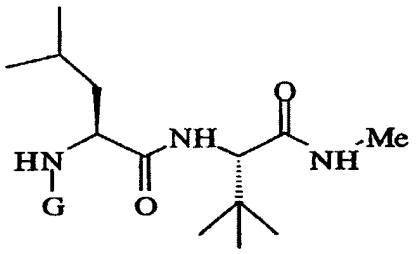
VERSION WITH MARKINGS TO SHOW CHANGES MADE

Please amend claims 1-13 as follows:

1. (Amended) [Process] A process for the preparation of a dipeptide of formula 1 comprising



(1)

[where G represents a protective group with] coupling N-protected L-leucine [being coupled] to L-*tert.*-leucine-N-methylamide in the presence of an activating agent, [characterized in that a formyl group is used as protective group] wherein G is a protective group that is a formyl group.

2. (Amended) [Process] The process according to claim 1 in which the L-*tert.*-leucine-N-methylamide has an enantiomeric excess greater than 98%

3. (Amended) [Process] The process according to claim 1 [or 2] in which the N-formyl-L-leucine has an enantiomeric excess greater than 98%.

4. (Amended) [Process] The process according to [any one of]claim[s] 1[-3] further comprising subjecting [in which] the N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide obtained [is subsequently subjected] to one or more crystallizations.

5. (Amended) [Process] The process according to [any one of]claim[s] 1[-4] further comprising deformylating [in which] the dipeptide obtained [is subsequently deformylated].

6. (Amended) [Process] The process according to claim 5 further comprising subjecting [in which] the L-leucyl-L-*tert.*-leucine-N-methylamide obtained [is subsequently subjected] to one or more crystallizations.

7. (Amended) [Process] The process according to claim 5 [or 6 in which] further comprising coupling the L-leucyl-L-*tert.*-leucine-N-methylamide [is subsequently coupled] to a substituted or nonsubstituted α-mercaptocarboxylic acid to form the corresponding N-α-optionally substituted mercaptocarboxyl-L-leucyl-L-*tert.*-leucine-N-methylamide.

8. (Amended) A compound which is N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide.

9. (Amended) A composition comprising the N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide defined in claim 8 wherein [with] an enantiomeric excess is present of the N-terminal amino acid in the dipeptide of more than 80%.

10. (Amended) The composition according to claim 9 wherein the [N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide with an] enantiomeric excess of the N-terminal amino acid in the dipeptide [of] is more than 98%.

11. (Amended) The composition [N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide] according to claim 9[or 10 with] wherein a diastereomeric excess is present of more than 80%.

12. (Amended) The composition [N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide] according to claim 11 with a diastereomeric excess of more than 98%.

13. (Amended) A pharmaceutical composition comprising [Use of] N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide according to [any one of]claim[s] 8[-12 in the preparation of pharmaceuticals] and a pharmaceutically acceptable excipient.

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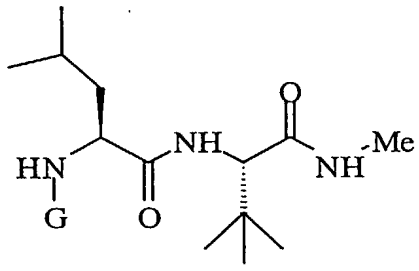
PROCESS FOR THE PREPARATION OF A DIPEPTIDE AND INTERMEDIATE PRODUCT IN SUCH A PROCESS

The invention relates to a process for the preparation of a dipeptide of formula 1



(1)

in which G represents a protective group, with N-protected L-leucine being coupled to L-*tert.*-leucine-N-methylamide in the presence of an activating agent.

WO-A-96/11209 discloses such a process in which N-(1,1-dimethylethoxy)carbonyl-L-leucine and L-*tert.*-leucine-N-methylamide are coupled.

A drawback of the known process is that it uses an expensive protective group, so that the process is less attractive from a commercial point of view. The present invention provides a commercially attractive route for the preparation of the above-mentioned intermediate product in the preparation of, for instance, the pharmaceuticals as described in WO-A-96/11209.

This is achieved according to the invention by using a formyl group as protective group.

Dipeptide couplings involving the coupling of two amino acids are generally known and are described

in detail in the literature. In these couplings the activated acid group of the eventual N-terminal amino acid reacts with the amino group of the eventual C-terminal amino acid or amino acid derivative. In this process the amino group of the eventual N-terminal amino acid is protected by means of a protective group.

In the process according to the invention two enantiomer-enriched amino acids are coupled. The enantiomeric excess of the enantiomer-enriched amino acids is preferably greater than 80%, in particular greater than 90%, more in particular greater than 98%. It is known that racemization of the N-terminal amino acid may take place when the amino acids are coupled. This is the case in particular when a formyl protective group is used, such as for instance described in the handbooks Houben-Weyl, Band 15/1 (1974), p. 166, and The Peptides, Academic Press 1979, Volume 1, p. 279. As a consequence, formyl protective groups are not considered for coupling of enantiomer-enriched amino acids. Applicant has now found that no racemization or only a low degree of racemization takes place when the coupling is carried out according to the invention, with a formyl group being used as protective group. Moreover, applicant has found that, should racemization take place, this very coupling product according to the invention is particularly suitable for enrichment in the desired diastereomeric form through crystallization.

An added advantage of the process according to the invention is that inexpensive activating agents can be used in the process.

The N-formyl-L-leucine that is used in the

process according to the invention can for instance be prepared in a known manner by contacting L-leucine with formic acid and for instance an anhydride. Preferably, use is made of acetic anhydride.

The L-*tert.*-leucine-N-methylamide can for instance be prepared from L-*tert.*-leucine via the conversion of L-*tert.*-leucine and phosgene into L-*tert.*-leucine-N-carboxyanhydride, which is subsequently converted into L-*tert.*-leucine-N-methylamide with the aid of N-methylamine.

In the process according to the invention the N-formyl-L-leucine is activated by means of an activating agent, preferably a sterically hindered acid chloride or an alkyl chloroformiate, and a base. Such activation steps are generally known and are often applied in peptide couplings. The bases to be used therefore are preferably the known bases used in these activation steps, with a low degree of racemization occurring. Preferably, N-methylmorpholine is used as base.

The temperature at which the activation is carried out is not very critical and in practice usually lies between -30°C and +30°C, preferably between -20°C and +10°C.

If desired the activation is carried out in a solvent, preferably one that is inert in the reaction mixture. Examples of solvents esters are esters, in particular ethyl acetate, isopropyl acetate and isobutyl acetate, ethers, in particular tetrahydrofuran (THF), methyl-*tert.*-butylether (MTBE) and dioxane, and nitriles, in particular acetonitrile.

In one embodiment first the activation is

carried out followed by a coupling step. For the coupling, the activated N-formyl-L-leucine is contacted with the L-*tert.*-leucine-N-methylamide. Preferably, a solution of L-*tert.*-leucine-N-methylamide is used.

In principle, for the temperature at which the coupling takes place the same holds as for the temperature at which the activation is carried out. Preferably, the coupling temperature is about the same as the activation temperature. Examples of suitable solvents for the L-*tert.*-leucine-N-methylamide are alcohols, in particular methanol, ethanol and isopropanol, esters, in particular ethyl acetate, isopropyl acetate and isobutyl acetate and ethers, in particular THF, MTBE and dioxane.

Alternatively a one stage procedure may be followed for the activation and the coupling, wherein the N-formyl-L-leucine, the L-*tert.*-leucine-N-methyl amide and the base are solved in a suitable solvent as described above, and the activating agent is added to the solution.

The resulting N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide can subsequently be deformylated in a generally known manner, for instance in an acid environment. The deformylation can for instance be carried out in an aqueous environment, in water/alcohol mixtures or in a two-phase system.

The temperature at which the deformylation is carried out for instance lies between 20°C and 110°C, preferably between 40°C and 80°C.

The resulting N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide or L-leucyl-L-*tert.*-leucine-N-methylamide can if desired be purified, for instance by

subjecting it to a crystallization. Surprisingly, it has been found that the enantiomeric excess of the N-terminal amino acid in the protected or non-protected dipeptide can be increased by the crystallization in those cases in which racemization has taken place during the peptide coupling.

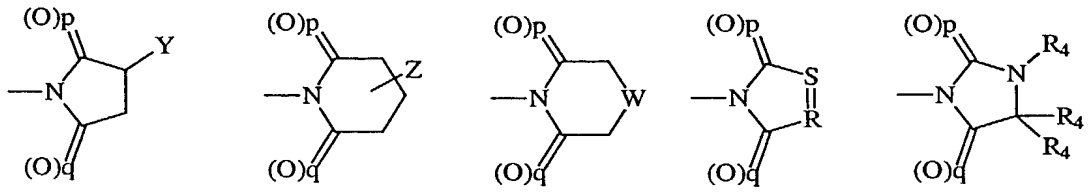
Examples of suitable solvents that can be used in the crystallization are hydrocarbons, in particular heptane and hexane; esters, in particular isopropyl acetate, isobutyl acetate and ethyl acetate; ethers, in particular MTBE; alcohols, in particular methanol, ethanol, isopropanol and butanol; or mixtures thereof. An example of a suitable mixture of solvents is a mixture of heptane and isopropyl acetate.

The temperature at which the crystallization is carried out is not particularly critical and depends mainly on the physical parameters of the chosen solvent, particularly the boiling point. In practice, the crystallization will usually be carried out at a temperature between 20°C and 100°C.

Depending on the exact embodiment of the peptide coupling, it may be advantageous to isolate the N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide intermediate product obtained, for instance via extraction or crystallization.

The L-leucyl-L-*tert.*-leucine-N-methylamide obtained can for instance be applied in the preparation of pharmaceuticals, for instance the N-(α-optionally substituted mercaptocarboxyl)- L-leucyl-L-*tert.*-leucine-N-methylamide compounds such as described in WO-A-96/11209 and WO-A-97/12902. The α-optionally substituted mercaptocarboxyl group for instance

represents a group of formula $R_1S-C(R_2)-C(O)-$ where R_1 stands for H or R_3CO where R_3 is a C_{1-4} alkyl, (C_{1-4} alkyl)aryl group, (C_{1-6} alkyl)heteroaryl group, C_{3-6} cycloalkyl) group, C_{3-6} cycloalkyl)C_{1-4} alkyl group, C_{2-6} alkenyl group, (C_{2-6} alkenyl) aryl group, aryl group or heteroaryl group; and R_2 stands for H or a C_{1-4} alkyl-C(O)-A- or C_{1-4} alkyl-NH-C(O)-A group, where A stands for



p and q are each independently 0 or 1

R_4 = H or a C_{1-6} alkyl group (each R_4 independent of the other one)

Y and Z are each independently H or (C_{0-4} alkyl)R_5, where R_5 is NHR_4, $N(R_4)_2$ (R_4 each independently), $COOR_4$, $CONHR_4$, $NHCO_2R_4$, $NHSO_2R_4$ or $NHCOR_4$ and

W is O, $S(O)_m$, with m = 0, 1 or 2, or NR_6

R_6 = H, C_{1-4} alkyl, COR_7, CO_2R_7, $CONHR_7$ or SO_2R_7

R_7 = H, C_{1-4} alkyl, aryl, heteroaryl, (C_{1-4} alkyl)aryl or (C_{1-4} alkyl) heteroaryl

R and S are each independently CH or N.

These compounds can be prepared in a known manner by for instance activating a substituted or non-substituted α-mercaptocarboxylic acid and coupling it to the L-leucyl-L-*tert.*-leucine-N-methylamide dipeptide obtained according to the invention using classical peptide coupling techniques, as for instance described in WO-A-96/11209 and WO-A-97/12902.

The invention will now be elucidated on the basis of examples, without however being restricted thereto.

Example I

Preparation of N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide from N-formyl-L-leucine and L-*tert.*-leucine-N-methylamide

Under nitrogen at -18°C isobutylchloroformiate (6.5 g, 48 mmol) was dosed to a solution of N-formyl-L-leucine (8.0 g, 50 mmol) in tetrahydrofuran (125 ml). Then N-methyl morpholine (4.8 g, 48 mmol) was added dropwise at such a rate that the temperature remained < -15°C. A precipitate was formed.

After stirring had been continued for 15 minutes, a solution of L-*tert.*-leucine-N-methylamide (6.5 g, 45 mmol) in tetrahydrofuran (50 ml) was added in such a way that the temperature remained < -15°C. Subsequently, stirring was continued for 1 hour at -18°C.

The reaction mixture was heated to 0°C and at this temperature water was added (100 g). Then THF was removed by distillation under vacuum. Isopropyl acetate (75 ml) was added and the pH of the reaction mixture was adjusted to 1.5 using hydrochloric acid. After layer separation, the aqueous phase was twice extracted with 50 and 35 ml isopropyl acetate, respectively. The collected organic phases were then washed with 50 and 25 ml saturated sodium bicarbonate solution and finally with 25 ml water. The organic phase was then evaporated under vacuum.

N-formyl-L-leucyl-L-*tert.*-leucine-N-

methylamide was obtained in a good yield and with an e.e. (L-leucine fragment) of 99% (HPLC).

Example II

Preparation of L-leucyl-L-*tert.*-leucine-N-methylamide from N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide

11.7 g (41 mmol) N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide (see Example I) was suspended in 1M HCl (100 ml) and heated to 40°C. After 18 hours' stirring at this temperature (all material went into solution), cooling to room temperature and one extraction with 50 ml isopropyl acetate took place.

After layer separation the pH of the aqueous phase was adjusted to 10 using 50% sodium hydroxide solution. Two extractions with isopropyl acetate (75 ml) were performed. The collected organic phases were evaporated under vacuum.

The residue was suspended in heptane (75 ml) and heated to 65°C. So much isopropyl acetate was added that everything just dissolved. After crystallization by means of cooling to room temperature and filtration, the material was washed twice with heptane (25 ml) and dried. L-leucyl-L-*tert.*-leucine-N-methylamide was obtained in a good yield with

purity = >98% (HPLC)
e.e. (L-leucine fragment) = 99% (HPLC)

Example III

Preparation of N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide from N-formyl-L-leucine and L-*tert.*-leucine-N-methylamide

Under nitrogen at -15°C

isobutylchloroformiate (12.3 g, 90 mmol) was dosed to a suspension of N-formyl-L-leucine (15.9 g, 100 mmol) in isopropyl acetate (85 ml). Subsequently, N-methyl morpholine (9.1 g, 90 mmol) in isopropylacetate (25ml) was added dropwise at such a rate that the temperature remained < -10°C.

After stirring had been continued for 90 minutes, the suspension formed was dosed to a cooled solution of L-*tert.*-leucine-N-methylamide (13.0 g, 90 mmol) in methanol (65 ml) in such a way that the temperature remained < -10°C. Stirring was subsequently continued for 30 minutes at -10°C.

The reaction mixture was heated to room temperature and further stirred at this temperature for 2 hours. 100 ml water was added to the reaction mixture and the pH was adjusted to 1.0 using 37% aqueous hydrochloride solution. After layer separation the aqueous phase was rewashed with two times 75 ml isopropyl acetate. The collected organic phases were then washed with 100 and 50 ml saturated sodium carbonate solution, respectively.

The organic phase was then evaporated under vacuum. N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide was obtained with an e.e. (L-leucine fragment) of 98% (HPLC).

Example IV

Preparation of N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide from N-formyl-L-leucine and L-*tert.*-leucine-N-methylamide

N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide was prepared as described in Example III,

but now at temperatures between 0-5°C. N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide was obtained with an e.e. (L-leucine fragment) of 86% (HPLC).

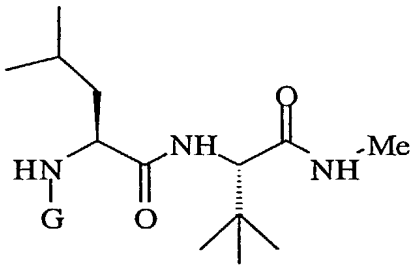
Example V

Preparation of L-leucyl-L-*tert.*-leucine-N-methylamide from N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide

The material obtained in Example IV was treated as described in Example II. L-leucyl-L-*tert.*-leucine-N-methylamide was obtained with an e.e. (L-leucine fragment) of 95% (HPLC).

C L A I M S

1. Process for the preparation of a dipeptide of formula 1



where G represents a protective group with N-protected L-leucine being coupled to L-*tert.*-leucine-N-methylamide in the presence of an activating agent, characterized in that a formyl group is used as protective group.

2. Process according to claim 1 in which the L-*tert.*-leucine-N-methylamide has an enantiomeric excess greater than 98%

3. Process according to claim 1 or 2 in which the N-formyl-L-leucine has an enantiomeric excess greater than 98%.

4. Process according to any one of claims 1-3 in which the N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide obtained is subsequently subjected to one or more crystallizations.

5. Process according to any one of claims 1-4 in which the dipeptide obtained is subsequently deformylated.

6. Process according to claim 5 in which the L-leucyl-L-*tert.*-leucine-N-methylamide obtained is

subsequently subjected to one or more crystallizations.

7. Process according to claim 5 or 6 in which the L-leucyl-L-*tert.*-leucine-N-methylamide is subsequently coupled to a substituted or non-substituted α-mercaptocarboxylic acid to form the corresponding N-α-optionally substituted mercaptocarboxyl-L-leucyl-L-*tert.*-leucine-N-methylamide.

8. N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide.

9. N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide with an enantiomeric excess of the N-terminal amino acid in the dipeptide of more than 80%.

10. N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide with an enantiomeric excess of the N-terminal amino acid in the dipeptide of more than 98%.

11. N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide according to claim 9 or 10 with a diastereomeric excess of more than 80%.

12. N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide according to claim 11 with a diastereomeric excess of more than 98%.

13. Use of N-formyl-L-leucyl-L-*tert.*-leucine-N-methylamide according to any one of claims 8-12 in the preparation of pharmaceuticals.

ABSTRACT

Process for the preparation of an N-formyl-L-leucyl-L-*tert*.-leucine-N-methylamide in which N-formyl L-leucine is coupled to L-*tert*.-leucine-N-methylamide in the presence of an activating agent. Preferably, use is made of L-*tert*.-leucine-N-methylamide with an enantiomeric excess greater than 98% and N-formyl-L-leucine with an enantiomeric excess greater than 98%. If desired, the dipeptide obtained is subsequently deformylated and the resulting N-formyl-L-leucyl-L-*tert*.-leucine-N-methylamide or the L-leucyl-L-*tert*.-leucine-N-methylamide is further subjected to one or more crystallizations.

The invention also relates to the N-formyl-L-leucyl-L-*tert*.-leucine-N-methylamide and the use of N-formyl-L-leucyl-L-*tert*.-leucine-N-methylamide in the preparation of pharmaceuticals.

PATENT
Docket No. 24615_2016400
3956US/CNT/1/

DECLARATION FOR [UTILITY/DESIGN] PATENT APPLICATION

AS A BELOW-NAMED INVENTOR, I HEREBY DECLARE THAT:

My residence, post office address, and citizenship are as stated below next to my name.

I believe I am the original, first and *[sole/joint] inventor of the subject matter which is claimed and for which a patent is sought on the invention entitled:

PROCESS FOR THE PREPARATION OF A DIPEPTIDE AND INTERMEDIATE PRODUCT IN SUCH A PROCESS,

the specification of which is attached hereto unless the following box is checked:

☒ was filed on 8 September 2000 as PCT International Application No. PCT/NL00/00635 and was amended on * (if applicable).

I HEREBY STATE THAT I HAVE REVIEWED AND UNDERSTAND THE CONTENTS OF THE ABOVE-IDENTIFIED SPECIFICATION, INCLUDING THE CLAIMS, AS AMENDED BY ANY AMENDMENT REFERRED TO ABOVE.

I acknowledge the duty to disclose information which is material to the patentability as defined in 37 C.F.R. § 1.56.

I hereby claim foreign priority benefits under 35 U.S.C. § 119(a)-(d) or § 365(b) of any foreign application(s) for patent or inventor's certificate, or § 365(a) of any PCT International application which designated at least one country other than the United States listed below and have also identified below, by checking the box, any foreign application for patent or inventor's certificate, or PCT International application having a filing date before that of the application on which priority is claimed:

Application No.	Country	Date of Filing (day/month/year)	Priority Claimed?
1013404	The Netherlands	27/10/1999	☒Yes ☐No

I hereby claim benefit under 35 U.S.C. § 119(e) of any United States provisional application(s) listed below:

Application Serial No.	Filing Date
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I hereby claim the benefit under 35 U.S.C. § 120 of any United States application(s), or § 365(c) of any PCT International application designating the United States, listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior

United States or PCT International application in the manner provided by the first paragraph of 35 U.S.C. § 112, I acknowledge the duty to disclose information which is material to patentability as defined in 37 C.F.R. § 1.56 which became available between the filing date of the prior application and the national or PCT International filing date of this application.

Application Serial No.	Filing Date	Status
PCT/NL00/00635	08/09/2000	☐Patented ☒Pending ☐Abandoned

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I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under § 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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